

## AMENDMENTS TO THE CLAIMS

1. (Previously presented) A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:
  - (a) collecting whole blood;
  - (b) administering an anticoagulant to the whole blood;
  - (c) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
  - (d) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA;
  - (e) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA; and
  - (f) quantifying the specific mRNA.
2. (Cancelled)
3. (Original) The method of Claim 1, wherein heparin is administered to the whole blood prior to collection of leukocytes.
4. (Original) The method of Claim 1, wherein the whole blood is frozen prior to filtration.
5. (Original) The method of Claim 1, wherein the filter membrane is attached to a multi-well filter plate.
6. (Original) The method of Claim 1, wherein the filter membrane is a PBT fibrous membrane.
7. (Original) The method of Claim 5, wherein the leukocytes are captured on a plurality of filter membranes layered together.
8. (Original) The method of Claim 1, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.
9. (Original) The method of Claim 8, additionally comprising drying the filter membrane.
10. (Original) The method of Claim 9, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

11. (Original) The method of Claim 1, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

12. (Original) The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

13. (Original) The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

14. (Original) The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

15. (Original) The method of Claim 1, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

16. (Original) The method of Claim 1, wherein the mRNA quantified is  $\beta$ -actin mRNA.

17. (Original) The method of Claim 1, wherein the mRNA quantified is CD4 mRNA.

18. (Original) The method of Claim 1, wherein the mRNA of a translocation gene involved in leukemia is quantified.

19. (Original) The method of Claim 1, wherein the mRNA of cancer-specific genes from micrometastatic cancer is quantified.

20. (Original) The method of Claim 1, wherein virus-derived mRNA from infected white blood cells is quantified.

21. (Original) The method of Claim 20, wherein the quantified virus-derived mRNA is HIV

22. (Original) The method of Claim 21, wherein the quantification of HIV mRNA is used to diagnose HIV.

23. (Original) The method of Claim 20, wherein the quantified virus-derived mRNA is CMV.

24. (Original) The method of Claim 23, wherein the quantification of virus-derived mRNA is used to diagnose CMV.

25. (Original) The method of Claim 20, wherein the quantification of virus-derived mRNA is used to monitor blood banks for the presence of viral diseases.

26. (Original) The method of Claim 20, wherein the quantification of virus-derived mRNA is used to study anti-viral drug sensitivity.

27. (Original) The method of Claim 1, wherein the mRNA of apoptosis genes involved in leukemia is quantified.

28. (Original) The method of Claim 1, wherein the mRNA of cytokines is quantified.

29. (Original) The method of Claim 1, wherein the quantification of mRNA is used to test side effects of anti-cancer drugs on white blood cells.

30. (Original) The method of Claim 1, wherein the mRNA of DNA-repair genes is quantified.

31. (Original) The method of Claim 30, wherein the quantification of mRNA of DNA-repair genes is used to test the sensitivity of DNA-repair genes to radiation.

32. (Original) The method of Claim 1, wherein the mRNA of allergen response genes is quantified.

33. (Original) The method of Claim 32, wherein the quantification of mRNA of allergen response genes is used to test allergen stimulation.

34. (Original) The method of Claim 1, wherein the mRNA of donor cell-mediated cytokines is quantified.

35. (Original) The method of Claim 34, wherein the quantification of mRNA of donor cell-mediated cytokines is used to test transplant rejection.

36. (Original) The method of Claim 1, additionally comprising determining the quantity of target mRNA in the sample using spiked control RNA.

37. (Original) The method of Claim 1, additionally comprising application of specific antisense primers during said lysate transferring step.

38. (Original) The method of Claim 1, additionally comprising application of specific antisense primers during said mRNA quantification step.

39. (Original) A high throughput mRNA quantification device, comprising:

- (a) a multi-well plate, said multi-well plate comprising:
  - i) a plurality of sample-delivery wells;
  - ii) a leukocyte-capturing filter underneath said wells;

Appl. No. : 10/796,298  
Filed : March 9, 2004

iii) an mRNA capture zone underneath said filter, said mRNA capture zone having oligo(dT)-immobilized thereon; and

(b) a vacuum box adapted to receive said plate to create a seal between said plate and said box.

40. (Original) The device of Claim 39, said vacuum box being adapted to receive a source of vacuum.

41. (Original) The device of Claim 39, said vacuum box being made of plastic.

42. (Original) The device of Claim 39, wherein said seal comprises a plastic-based gasket placed below the multi-well plate.

43. (Original) The device of Claim 39, wherein a multi-well supporter is inserted between the vacuum box and the multi-well plate.

44. (Original) The device of Claim 39, wherein the leukocytes are captured on a plurality of filter membranes layered together.

45. (Original) A lysis buffer for high throughput mRNA quantification, comprising:

(a) a sufficient concentration of detergent to lyse a cytoplasmic membrane;  
(b) a sufficient concentration of salt that the stringency does not exceed that of

4X SSC;

(c) a buffer to maintain pH in the range of 7.0-8.0;  
(d) 1.4-1.75 M guanine thiocyanate; and  
(e) 200 µg/ml -20 mg/ml proteinase K.

46. (Original) The lysis buffer of Claim 45, wherein the concentration of detergent is sufficient to lyse both cytoplasmic and nuclear membranes.

47. (Original) The lysis buffer of Claim 45, wherein the detergent comprises a plurality of detergents.

48. (Original) The lysis buffer of Claim 45, wherein the detergent comprises 0.1-2% IGEPAL CA-630.

49. (Original) The lysis buffer of Claim 45, wherein the detergent comprises 0.05-2% N-Lauroylsarcosine.

**Appl. No.** : **10/796,298**  
**Filed** : **March 9, 2004**

50. (Original) The lysis buffer of Claim 45, wherein the buffer is sufficient to maintain pH in the range of 7.4-8.0.

51. (Original) The lysis buffer of Claim 50, wherein the buffer comprises 1 mM-100 mM Tris HCL.

52. (Original) The lysis buffer of Claim 45, comprising about 1.6 M to about 1.7 M guanidine thiocyanate.

53. (Original) The lysis buffer of Claim 45, comprising 200  $\mu$ g/ml -1.0 mg/ml proteinase K

54. (Original) The lysis buffer of Claim 45, comprising 200  $\mu$ g/ml -500 $\mu$ g/ml proteinase K.

55. (Original) The lysis buffer of Claim 45, further comprising a chelating agent in an amount sufficient to chelate  $Mg^{2+}$  and  $Ca^{2+}$ .

56. (Original) The lysis buffer of Claim 55, wherein the chelating agent comprises 0.1-5 mM EDTA.

57. (Original) The lysis buffer of Claim 45, further comprising 0.1-10% 2-mercaptoethanol.

58. (Original) The lysis buffer of Claim 45, further comprising DNA.

59. (Original) The lysis buffer of Claim 58, wherein the DNA comprises 10 mg/ml sonicated salmon sperm DNA.

60. (Original) The lysis buffer of Claim 45, further comprising tRNA.

61. (Original) The lysis buffer of Claim 60, wherein the tRNA comprises 10 mg/ml E. Coli tRNA.

62. (Original) The lysis buffer of Claim 45, further comprising spiked control RNA.

63. (Original) The lysis buffer of Claim 62, wherein the spiked control RNA is selected from the group consisting of SEQ ID NOS 34, 36, and *bcr-abl* RNA.

64. (Original) The lysis buffer of Claim 62, wherein the spiked control RNA comprises poly(A)<sup>+</sup> RNA.

65. (Original) The lysis buffer of Claim 45, further comprising specific antisense primers.

66. (Original) A high throughput mRNA quantification kit, comprising:

- (a) the high throughput mRNA quantification device of Claim 36;
- (b) a hypotonic buffer;
- (c) ethanol; and
- (d) a lysis buffer.

67. (Original) The kit of Claim 66, wherein the lysis buffer comprises 1.4-1.75 M guanine thiocyanate; and 200 µg/ml -20 mg/ml proteinase K.

68. (Original) The kit of Claim 67, wherein the lysis buffer further comprises sufficient detergent to lyse a cytoplasmic membrane; sufficient salt that the stringency does not exceed that of 4X SSC; and a buffer to maintain pH in the range of 7.0-8.0.

69. (Original) The kit of Claim 67, wherein the lysis buffer further comprises sufficient salt that the stringency does not exceed that of 4X SSC.

70. (Original) The kit of Claim 67, wherein the lysis buffer further comprises sufficient buffer to maintain pH in the range of 7.0-8.0.

71. (Original) A method of lysing cells, comprising exposing cells to the lysis buffer of Claim 45.

72. (Original) A method of determining a definite quantity of leukocyte specific mRNA per µL of whole blood, comprising exposing cells to the lysis buffer of Claim 62.

73. (Previously presented) A method of determining a definite quantity of target mRNA in a blood sample comprising:

- (a) collecting whole blood;
- (b) administering an anticoagulant to the whole blood;
- (c) removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes;
- (d) lysing the leukocytes with a lysis buffer containing spiked control RNA to produce a lysate comprising mRNA and spiked control RNA;
- (e) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA;
- (f) quantifying the sample mRNA and spiked control RNA;
- (g) determining the percent recovery of spiked control RNA; and

(h) determining the definite quantity of mRNA by applying the percent recovery determined in step (g).

74. (Original) The method of Claim 73, wherein the spiked control RNA is not homologous to RNA present in the blood sample.

75. (Original) The method of Claim 73, wherein step (b) comprises filtration to yield leukocytes on a filter membrane.

76. (Cancelled)

77. (Original) The method of Claim 73, wherein heparin is administered to the whole blood prior to collection of leukocytes.

78. (Original) The method of Claim 73, wherein the whole blood is frozen prior to filtration.

79. (Original) The method of Claim 75, wherein the filter membrane is attached to a multi-well filter plate.

80. (Original) The method of Claim 79, wherein 10 to  $1e^{10}$  copies of spiked control RNA are applied to each filterplate.

81. (Original) The method of Claim 79, wherein  $1e^5$  to  $1e^{10}$  copies of spiked control RNA are applied to each filterplate.

82. (Original) The method of Claim 75, wherein the filter membrane is a PBT fibrous membrane.

83. (Original) The method of Claim 75, wherein the leukocytes are captured on a plurality of filter membranes layered together.

84. (Original) The method of Claim 75, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.

85. (Original) The method of Claim 84, additionally comprising drying the filter membrane.

86. (Original) The method of Claim 85, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

87. (Original) The method of Claim 73, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

**Appl. No.** : **10/796,298**  
**Filed** : **March 9, 2004**

88. (Original) The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

89. (Original) The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

90. (Original) The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

91. (Original) The method of Claim 73, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

92. (Original) The method of Claim 73, additionally comprising application of specific antisense primers during said lysate transferring step.

93. (Original) The method of Claim 73, additionally comprising application of specific antisense primers during said mRNA quantification step.

94. (Original) A method of synthesizing cDNA in solution upon poly-A RNA, comprising application of specific antisense primers during hybridization of RNA poly-A tails and immobilized oligo(dT).

95. (Original) A method of synthesizing cDNA in solution upon poly-A RNA, comprising application of specific antisense primers during cDNA synthesis.

96. (Original) A method for quantifying a first specific mRNA comprising a particular sequence from a sample, comprising:

- a) spiking said sample with a known quantity of a second specific mRNA;
- b) purifying poly-A mRNA from the sample;
- c) producing cDNA from the mRNA in the sample;
- d) quantifying an amount of cDNA corresponding to each of the first and second specific mRNA's in the sample;
- e) determining a percent recovery of the second specific mRNA; and
- f) applying the percent recovery of the second specific mRNA to determine the starting amount of the first specific mRNA.

97. (Original) The method of Claim 96, additionally comprising quantifying a third specific mRNA by a method comprising:

- a) spiking said sample with a known quantity of a second specific mRNA;

**Appl. No.** : **10/796,298**  
**Filed** : **March 9, 2004**

- b) purifying poly-A mRNA from the sample;
- c) producing cDNA from the mRNA in the sample;
- d) quantifying an amount of cDNA corresponding to each of the third and second specific mRNA's in the sample;
- e) determining a percent recovery of the second specific mRNA; and
- f) applying the percent recovery of the second specific mRNA to determine the starting amount of the third specific mRNA.

98. (Original) The method of Claim 96, wherein the sequence of the second specific mRNA is dissimilar to the first specific mRNA.

99. (Original) The method of Claim 98, wherein the sequence of the second specific mRNA is less than 90% homologous to or has at least a 10% difference in length from the first specific mRNA.

100. (Original) The method of Claim 98, wherein the sequence of the second specific mRNA is less than 85% homologous to or has at least a 5% difference in length from the first specific mRNA.

101. (Original) The method of Claim 98, wherein the sequence of the second specific mRNA is less than 75% homologous to or has at least a 2% difference in length from the first specific mRNA.

102. (Original) The method of Claim 98, wherein the sequence of the second specific mRNA is less than 65% homologous to or has at least a 1% difference in length from the first specific mRNA.

103. (Original) The method of Claim 96, further comprising providing a plurality of different first specific mRNAs comprising dissimilar sequences.

104. (Original) The method of Claim 96, further comprising providing a plurality of different second specific mRNAs comprising dissimilar sequences.

105. (Original) The method of Claim 104, further comprising providing a plurality of different first specific mRNAs comprising dissimilar sequences.

106. (Original) The method of Claim 96, wherein the purification of poly-A mRNA from the sample comprises hybridizing the poly-A to oligo(dT).

107. (Original) The method of Claim 106, wherein the oligo(dT) is immobilized.

Appl. No. : 10/796,298  
Filed : March 9, 2004

108. (Original) The method of Claim 96, wherein the second specific mRNA is added to the sample prior to purification.

109. (Original) The method of Claim 96, wherein the sample comprises whole blood.

110. (Original) The method of Claim 109, further comprising adding the sample and second specific mRNA to wells of a filtration device.

111. (Original) The method of Claim 110, wherein the purification of poly-A mRNA from the sample comprises applying lysis buffer to the wells.

112. (Original) The method of Claim 111, wherein the filtration device is a filter membrane.

113. (Original) The method of Claim 112, additionally comprising removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes.

114. (Original) The method of Claim 113, wherein the whole blood is frozen prior to filtration.

115. (Original) The method of Claim 113, wherein an anticoagulant is administered to the sample prior to filtration.

116. (Original) The method of Claim 113, wherein the filter membrane is attached to a multi-well filter plate.

117. (Original) The method of Claim 113, wherein 10 to  $1e^{10}$  copies of spiked control RNA are applied to each filterplate.

118. (Original) The method of Claim 113, wherein  $1e^5$  to  $1e^{10}$  copies of spiked control RNA are applied to each filterplate.

119. (Original) The method of Claim 113, wherein the filter membrane is a PBT fibrous membrane.

120. (Original) The method of Claim 113, wherein the leukocytes are captured on a plurality of filter membranes layered together.

121. (Original) The method of Claim 113, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.

**Appl. No.** : **10/796,298**  
**Filed** : **March 9, 2004**

122. (Original) The method of Claim 121, additionally comprising drying the filter membrane.

123. (Original) The method of Claim 122, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

124. (Original) The method of Claim 116, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

125. (Original) The method of Claim 124, additionally comprising transfer of lysate to the oligo(dT)-immobilized plate.

126. (Original) The method of Claim 125, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

127. (Original) The method of Claim 125, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

128. (Original) The method of Claim 125, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

129. (Original) The method of Claim 128, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

130. (Original) The method of Claim 129, additionally comprising application of specific antisense primers during said mRNA quantification step.

131. (Original) The method of Claim 96, wherein the production of cDNA from the mRNA comprises application of PCR primers to the first and second specific mRNAs.

132. (Original) The method of Claim 131, wherein the production of cDNA from the mRNA further comprises providing probe molecules.

133. (Original) The method of Claim 96, wherein the quantification of cDNA step comprises amplification of cDNA.

134. (Original) The method of Claim 96, wherein the amplification comprises PCR.

135. (Original) The method of Claim 96, wherein the amplification comprises real time PCR.

136. (Original) A method of detecting mRNA indicative of a cellular response to a bioagent, comprising:

Appl. No. : 10/796,298  
Filed : March 9, 2004

- a) exposing cells to a bioactive agent;
- b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
- d) purifying poly-A mRNA from the lysate;
- e) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
- f) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
- g) creating a graph comprising the amount of recovered specific native mRNA on the y axis and the recovered specific control mRNA on the x axis; and
- h) using the graph of step (g) to determine the amount of mRNA produced in response to exposure to a bioactive agent.

137. (Currently amended) The method of Claim 162136, further comprising creating a graph comprising the amount of native mRNA on the y-axis and the source of the mRNA on the x axis.

138. (Currently amended) The method of Claim 162136, wherein the purification of poly-A mRNA from the sample comprises hybridizing the poly-A to oligo(dT).

139. (Currently amended) The method of Claim 163138, wherein the oligo(dT) is immobilized.

140. (Currently amended) The method of Claim 162136, further comprising adding the cells and lysis buffer to wells of a filtration device.

141. (Currently amended) The method of Claim 166140, wherein the filtration device is a filter membrane.

142. (Currently amended) The method of Claim 167141, wherein the filter membrane is attached to a multi-well filter plate.

143. (Currently amended) The method of Claim 168142, wherein 10 to  $1e^{10}$  copies of control RNA are applied to each filterplate.

144. (Currently amended) The method of Claim 162142, wherein  $1e^5$  to  $1e^{10}$  copies of control RNA are applied to each filterplate.

Appl. No. : 10/796,298  
Filed : March 9, 2004

145. (Currently amended) The method of Claim 169143, wherein the filter membrane is a PBT fibrous membrane.

146. (Currently amended) The method of Claim 171145, wherein the cells are captured on a plurality of filter membranes layered together.

147. (Currently amended) The method of Claim 172146, additionally comprising washing the cells on the filter membrane with hypotonic buffer.

148. (Currently amended) The method of Claim 173147, additionally comprising drying the filter membrane.

149. (Currently amended) The method of Claim 174148, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

150. (Currently amended) The method of Claim 175149, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

151. (Currently amended) The method of Claim 176150, additionally comprising transfer of lysate to the oligo(dT)-immobilized plate.

152. (Currently amended) The method of Claim 177151, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

153. (Currently amended) The method of Claim 177151, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

154. (Currently amended) The method of Claim 177151, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

155. (Currently amended) The method of Claim 162136, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

156. (Currently amended) The method of Claim 162136, additionally comprising application of specific antisense primers during said mRNA quantification step.

157. (Currently amended) The method of Claim 162136, wherein the production of cDNA from the mRNA comprises application of PCR primers to the first and second specific mRNAs.

158. (Currently amended) The method of Claim 183157, wherein the production of cDNA from the mRNA further comprises providing probe molecules.

159. (Currently amended) The method of Claim 162136, wherein the quantification of cDNA step comprises amplification of cDNA.

160. (Currently amended) The method of Claim 162159, wherein the amplification comprises PCR.

161. (Currently amended) The method of Claim 162159, wherein the amplification comprises real time PCR.

162. (Original) A method of detecting mRNA indicative of a cellular response to a bioagent, comprising:

- a) exposing cells to a bioactive agent;
- b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
- c) purifying poly-A mRNA from the lysate;
- d) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
- e) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
- f) creating a graph comprising the amount of recovered specific native mRNA on the y axis and the recovered specific control mRNA on the x axis; and
- g) comparing statistical differences among multiple points on the graph of step (f) to detect mRNA produced in response to exposure to a bioactive agent.

163. (Original) The method of Claim 162, further comprising creating a graph comprising the amount of native mRNA on the y-axis and the source of the mRNA on the x axis.

164. (Original) The method of Claim 162, wherein the purification of poly-A mRNA from the sample comprises hybridizing the poly-A to oligo(dT).

165. (Currently amended) The method of Claim 163164, wherein the oligo(dT) is immobilized.

166. (Original) The method of Claim 162, further comprising adding the cells and lysis buffer to wells of a filtration device.

167. (Original) The method of Claim 166, wherein the filtration device is a filter membrane.

Appl. No. : 10/796,298  
Filed : March 9, 2004

168. (Original) The method of Claim 167, wherein the filter membrane is attached to a multi-well filter plate.

169. (Original) The method of Claim 168, wherein 10 to  $1e^{10}$  copies of control RNA are applied to each filterplate.

170. (Currently amended) The method of Claim ~~162~~168, wherein  $1e^5$  to  $1e^{10}$  copies of control RNA are applied to each filterplate.

171. (Original) The method of Claim 169, wherein the filter membrane is a PBT fibrous membrane.

172. (Original) The method of Claim 171, wherein the cells are captured on a plurality of filter membranes layered together.

173. (Original) The method of Claim 172, additionally comprising washing the cells on the filter membrane with hypotonic buffer.

174. (Original) The method of Claim 173, additionally comprising drying the filter membrane.

175. (Original) The method of Claim 174, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

176. (Original) The method of Claim 175, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

177. (Original) The method of Claim 176, additionally comprising transfer of lysate to the oligo(dT)-immobilized plate.

178. (Original) The method of Claim 177, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

179. (Original) The method of Claim 177, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

180. (Original) The method of Claim 177, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

181. (Original) The method of Claim 162, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

182. (Original) The method of Claim 162, additionally comprising application of specific antisense primers during said mRNA quantification step.

Appl. No. : 10/796,298  
Filed : March 9, 2004

183. (Original) The method of Claim 162, wherein the production of cDNA from the mRNA comprises application of PCR primers to the first and second specific mRNAs.

184. (Original) The method of Claim 183, wherein the production of cDNA from the mRNA further comprises providing probe molecules.

185. (Original) The method of Claim 162, wherein the quantification of cDNA step comprises amplification of cDNA.

186. (Currently amended) The method of Claim ~~162~~<sup>185</sup>, wherein the amplification comprises PCR.

187. (Currently amended) The method of Claim ~~162~~<sup>185</sup>, wherein the amplification comprises real time PCR.

188. (Original) A method of identifying an individual expressing abnormal levels of mRNA:

- a) exposing cells to a bioactive agent;
- b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
- d) purifying poly-A mRNA from the lysate;
- e) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
- f) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
- g) creating a graph comprising the amount of recovered specific native mRNA on the y axis, the recovered specific control mRNA on the x axis, and a regression line;
- h) rotating the x-axis of the graph of step (g) to align with the regression line;
- i) determining the normal range of mRNA quantities from the graph of step (h); and
- j) detecting the individuals with mRNA quantities falling outside of the range of normal mRNA quantities.

189. (Original) The method of Claim 188, further comprising creating a graph comprising the amount of native mRNA on the y-axis and the source of the mRNA on the x axis.

Appl. No. : 10/796,298  
Filed : March 9, 2004

190. (Original) The method of Claim 188, wherein the purification of poly-A mRNA from the sample comprises hybridizing the poly-A to oligo(dT).
191. (Original) The method of Claim 189, wherein the oligo(dT) is immobilized.
192. (Original) The method of Claim 188, further comprising adding the cells and lysis buffer to wells of a filtration device.
193. (Original) The method of Claim 192, wherein the filtration device is a filter membrane.
194. (Original) The method of Claim 193, wherein the filter membrane is attached to a multi-well filter plate.
195. (Original) The method of Claim 194, wherein 10 to  $1e^{10}$  copies of control RNA are applied to each filterplate.
196. (Original) The method of Claim 188, wherein  $1e^5$  to  $1e^{10}$  copies of control RNA are applied to each filterplate.
197. (Original) The method of Claim 196, wherein the filter membrane is a PBT fibrous membrane.
198. (Original) The method of Claim 197, wherein the cells are captured on a plurality of filter membranes layered together.
199. (Original) The method of Claim 198, additionally comprising washing the cells on the filter membrane with hypotonic buffer.
200. (Original) The method of Claim 199, additionally comprising drying the filter membrane.
201. (Original) The method of Claim 200, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.
202. (Original) The method of Claim 201, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.
203. (Original) The method of Claim 202, additionally comprising transfer of lysate to the oligo(dT)-immobilized plate.
204. (Original) The method of Claim 203, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

**Appl. No.** : **10/796,298**  
**Filed** : **March 9, 2004**

205. (Original) The method of Claim 203, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

206. (Original) The method of Claim 203, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

207. (Original) The method of Claim 188, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

208. (Original) The method of Claim 188, additionally comprising application of specific antisense primers during said mRNA quantification step.

209. (Original) The method of Claim 188, wherein the production of cDNA from the mRNA comprises application of PCR primers to the first and second specific mRNAs.

210. (Original) The method of Claim 209, wherein the production of cDNA from the mRNA further comprises providing probe molecules.

211. (Original) The method of Claim 188, wherein the quantification of cDNA step comprises amplification of cDNA.

212. (Original) The method of Claim 188, wherein the amplification comprises PCR.

213. (Original) The method of Claim 188, wherein the amplification comprises real time PCR.

214. (New) A method of isolating mRNA from a cell lysate, comprising:  
    providing oligo(dT) polynucleotides immobilized on a solid support;  
    introducing a cell lysate comprising mRNA to said oligo(dT) polynucleotides, thereby creating a reaction mixture;  
    maintaining said reaction mixture at a temperature below 15°C for 60 minutes or more, thereby permitting hybridization of said mRNA and said oligo(dT) polynucleotides; and  
    removing all components of said cell lysate other than said hybridized mRNA.